

The Role of Microbial Phosphatases on Uranium Mobility in the Subsurface

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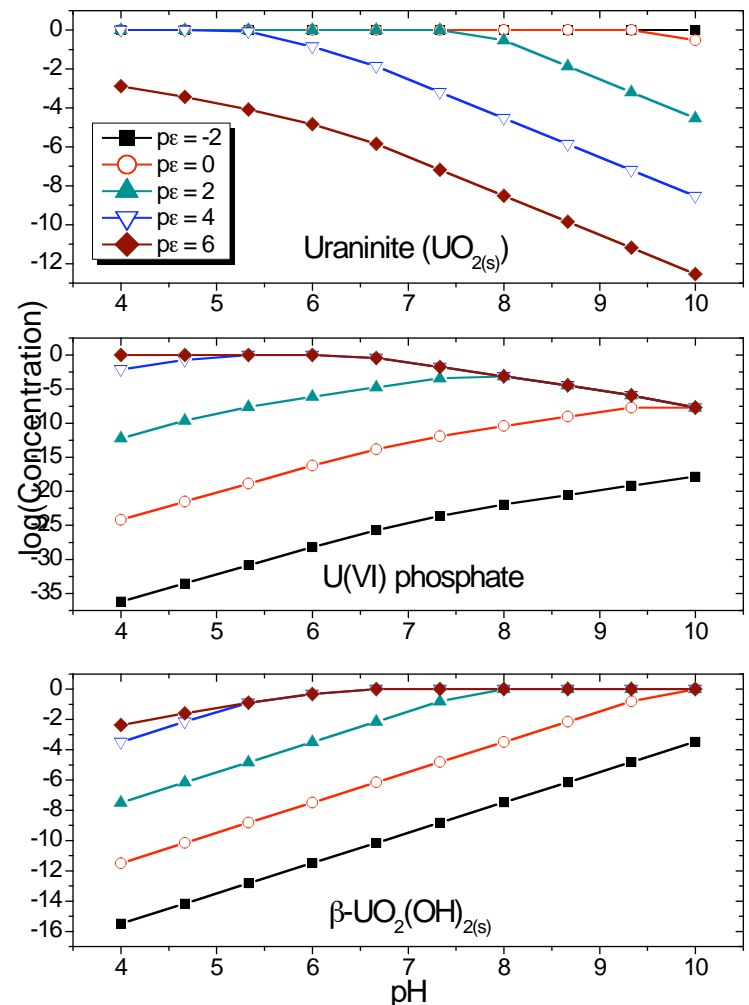
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Why U(VI) Phosphate?

- Apatites promote the precipitation of U(VI) phosphate in the subsurface (Arey et al., 1999; Fuller et al., 2002; Krestou et al., 2004; Ohnuki et al., 2004; Seaman et al., 2003; Wellman et al., 2008).
- U(VI) phosphate minerals are found in a variety of soils, including those of the ORFRC (Roh et al., 2000), Fernald (Morris et al., 1996), and Hanford sites (Arai et al., 2007).
- U(VI) phosphate minerals are extremely stable at pH > 3 (Ghafar et al., 2002; Krestou et al., 2004; Raicevic et al., 2006)
- U(VI) phosphate precipitation may complement U(VI) reduction in subsurfaces
 - Dissolved U is non-existent at pH < 7 at all p_e
 - At high p_e and pH, a maximum 10% of U is found as $\text{UO}_2(\text{HPO}_4)_2^{2-}$ complex

$\text{U}_T = 50 \mu\text{M}$; $\Sigma\text{PO}_4^{3-} = 40 \mu\text{M}$; $\Sigma\text{CO}_3^{2-} = 500 \mu\text{M}$

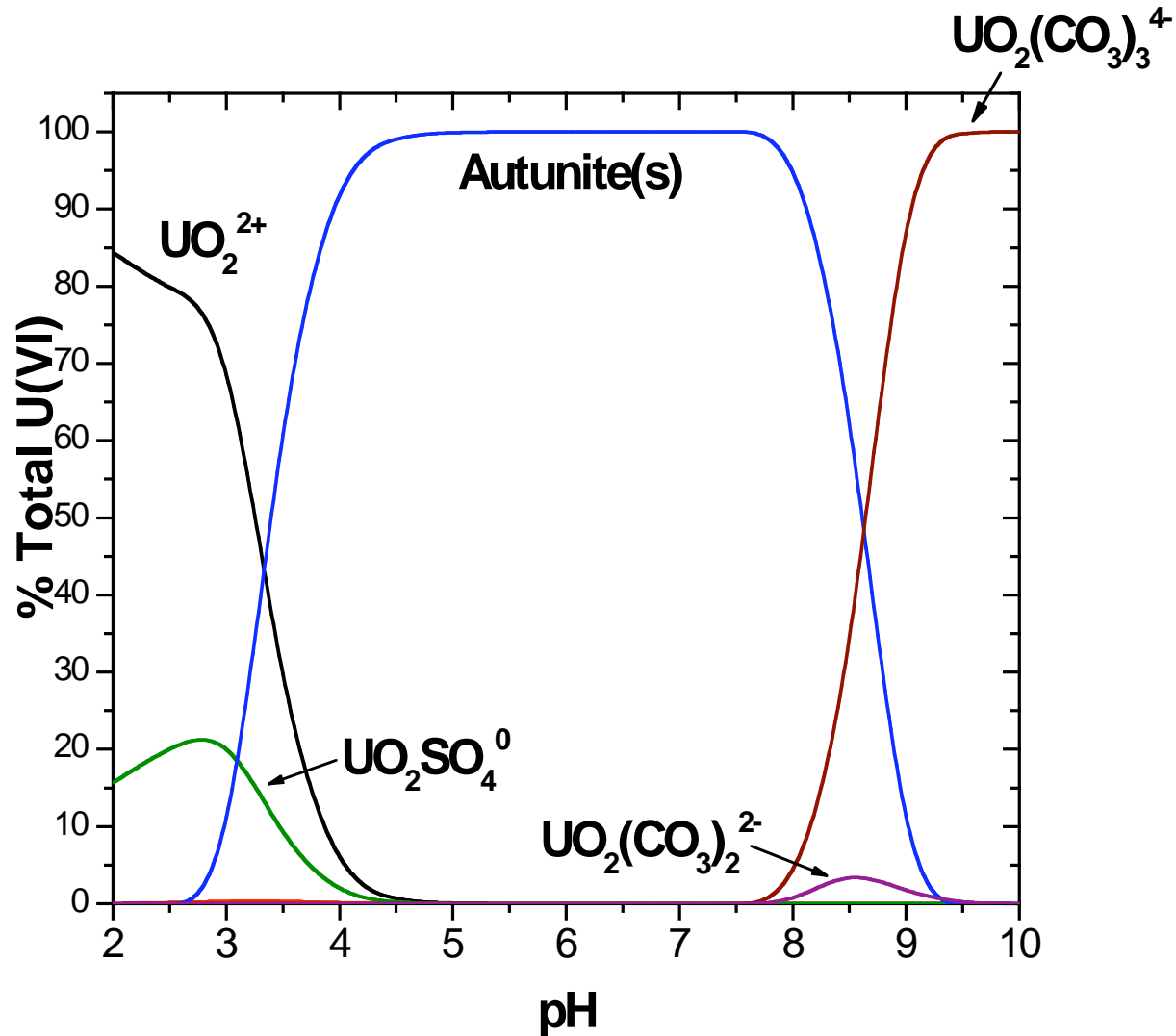


=> low pH and high p_e favor precipitation of U(VI) phosphates

=> high pH and high p_e favor precipitation of $\text{UO}_2(\text{OH})_{2(s)}$



Effect of pH on U(VI) Speciation

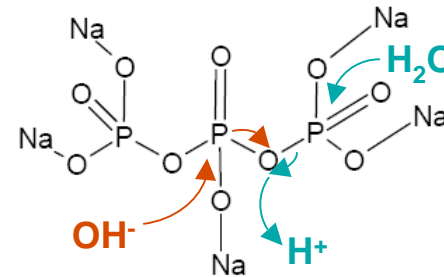
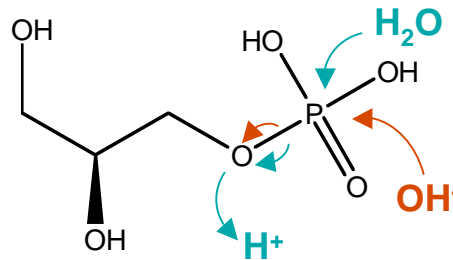


$\text{UO}_2^{2+} = 200 \mu\text{M}$, $\Sigma\text{PO}_4^{3-} = 1 \text{ mM}$, $P_{\text{CO}_2} = 10^{-3.5}$, simulated groundwater



Why U(VI) Phosphate?

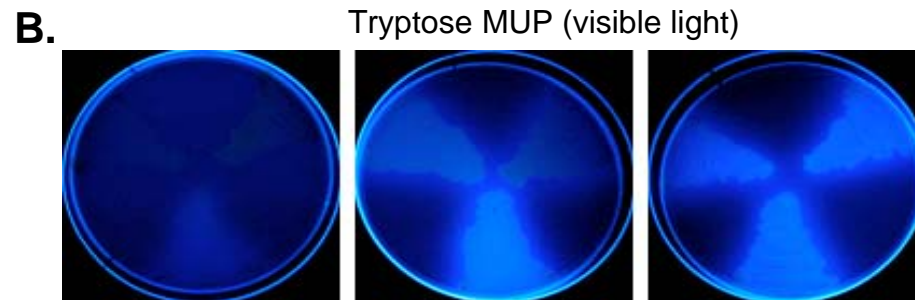
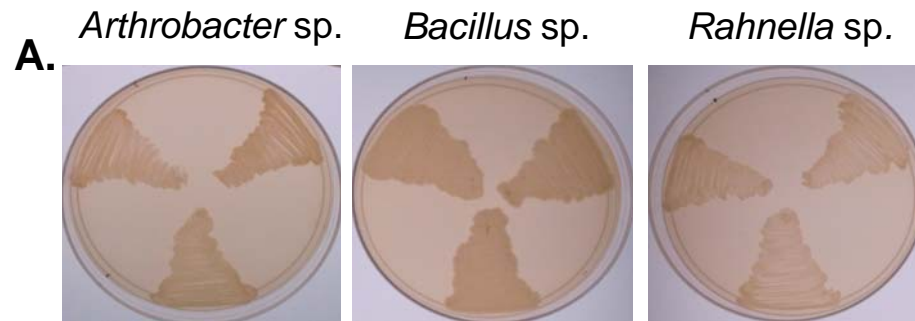
- Direct phosphate addition to groundwater is efficient in precipitating chernikovite but also decreases soil permeability (Wellman et al., 2006)
- Addition of organophosphate and polyphosphate compounds may promote the slow kinetics of uranium phosphate precipitation (Nash et al., 1998; Seaman et al., 2003; Wellman et al., 2006; Wellman et al., 2007)
- Mechanism: Hydrolysis



- Non-Specific Acid Phosphohydrolases (NSAP) mediate the release of phosphate from organophosphate sources to immobilize toxic metals (Macaskie et al., 1994), including uranium (Macaskie et al., 2000; Powers et al., 2002; Martinez et al., 2007).
- NSAPs exhibit optimal activity at acidic to neutral pH values and hydrolyze a broad array of organophosphoester compounds (Rossolini et al., 1998).
- **Objective:** Demonstrate that phosphatase activity of subsurface microbes results in the release of sufficient phosphate to precipitate U(VI)



Identifying Potential U-precipitating Phosphatase Phenotypes



Tryptose MUP (UV light)

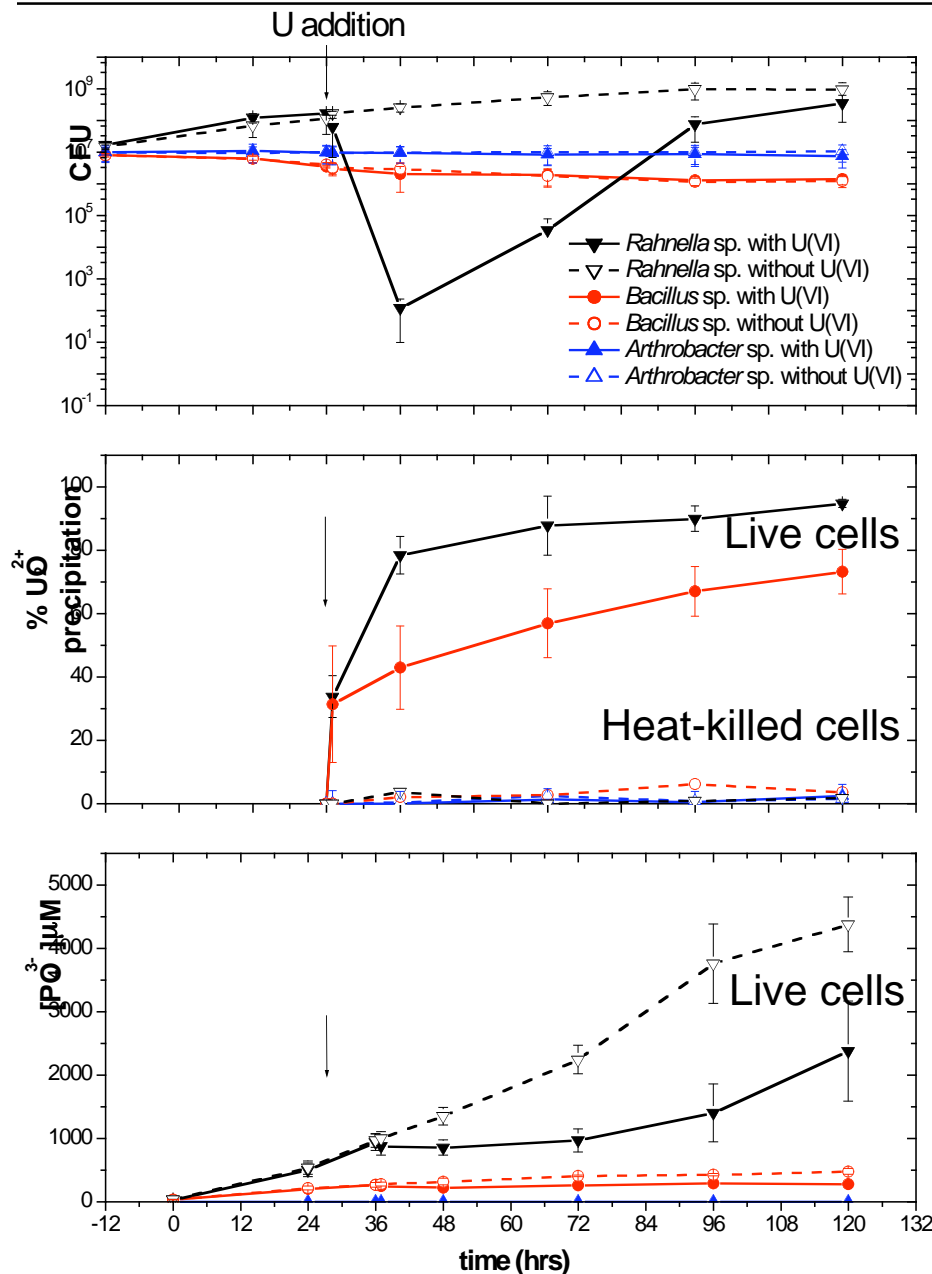
| Site Location | % Isolates with Phosphatase Phenotypes | % Isolates Resistant to Heavy Metals |
|---------------|--|--------------------------------------|
| Area 1 | 31 | 100 |
| Area 2 | 38 | 80 |
| Area 3 | 66 | 93 |
| Background | 12 | 100 |

- Heavy metal resistant subsurface isolates were selected
- Isolates were assayed for phosphatase activity.
- A significant percentage of isolates from contaminated soils (31-66%) exhibited phosphatase-positive phenotypes.
- A strong correlation between heavy metal resistance **and** phosphatase-positive phenotypes was noted.

Martinez et al., 2006 and 2007



Biomining of U(VI) through NSAP Activity



***Rahnella* sp., *Bacillus* sp., *Arthrobacter* sp.**

10 mM glycerol-3 phosphate (G3P)

200 μM U(VI) added after 36 hours

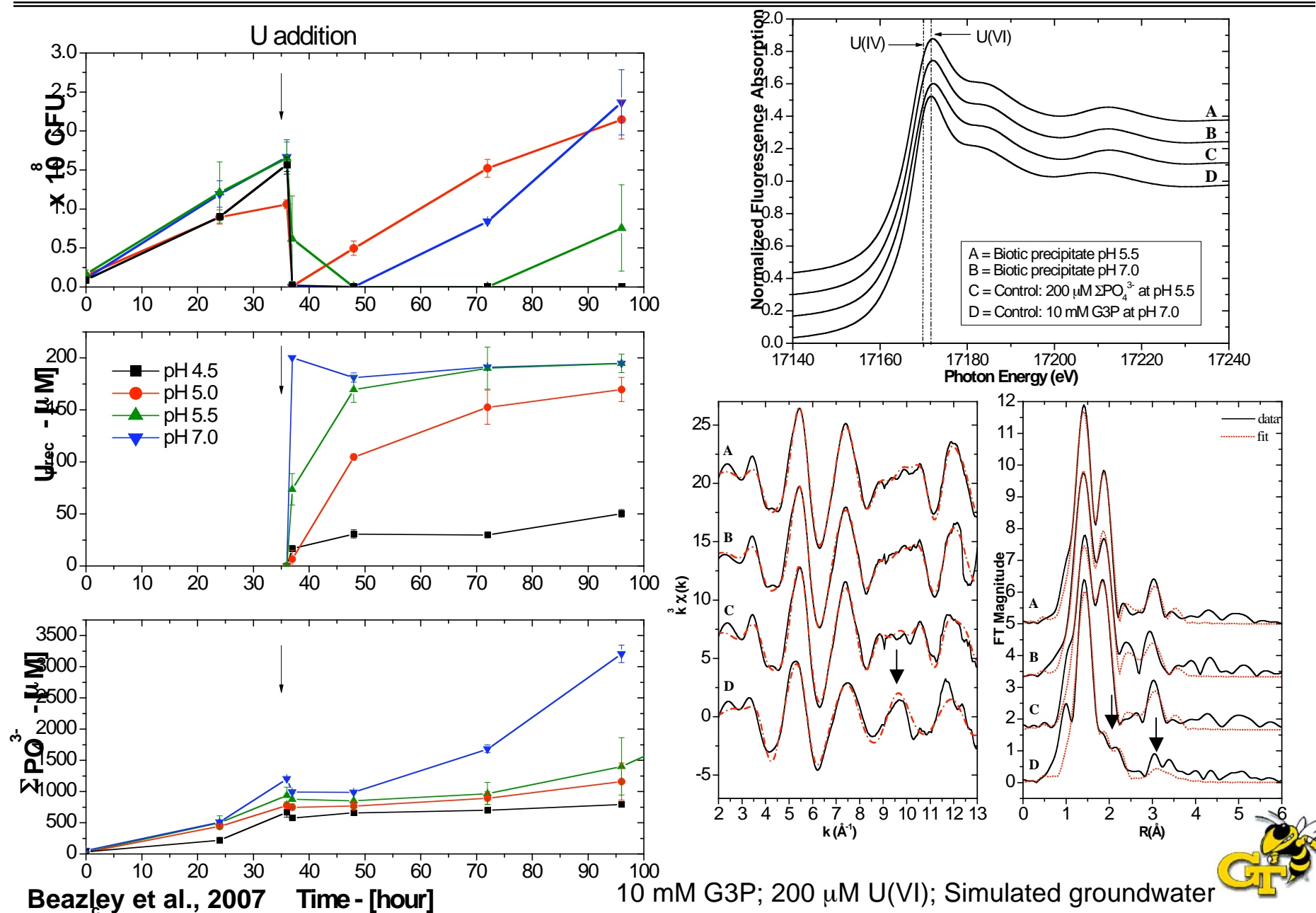
Simulated aerobic groundwater at pH 5.5

- *Rahnella* sp. grows easily, hydrolyzes G3P, and precipitates U(VI) the most efficiently
- *Bacillus* sp. hydrolyzes G3P and precipitate U(VI) but cells do not grow efficiently
- *Arthrobacter* sp. does not hydrolyze G3P and does not precipitate U(VI) but cells do not lose viability

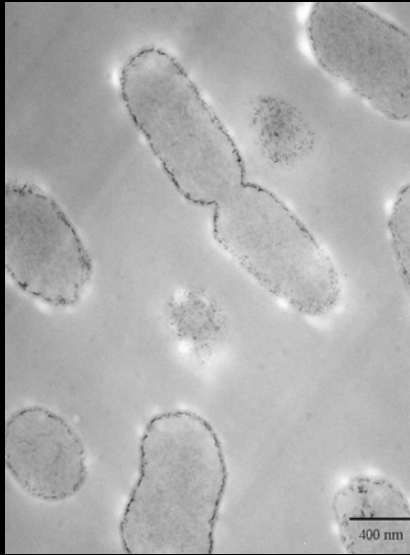
Martinez et al., 2007



Effect of pH on U(VI) precipitation by *Rahnella* sp.



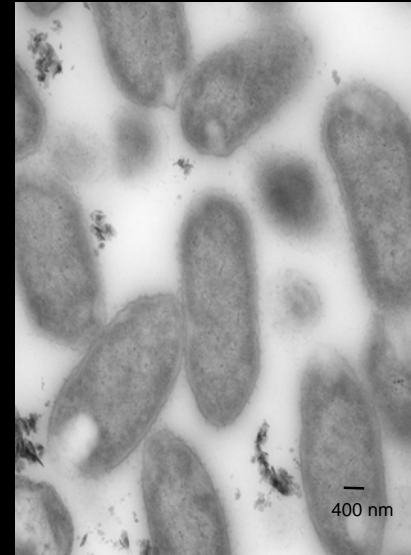
TEM images of *Rahnella* sp. during Incubation with G3P and U(VI)



U(VI) added; 1 hr

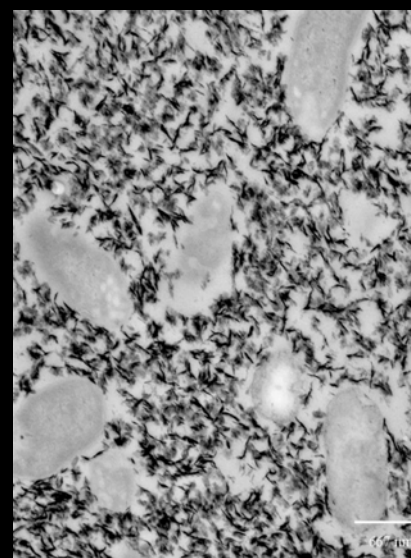
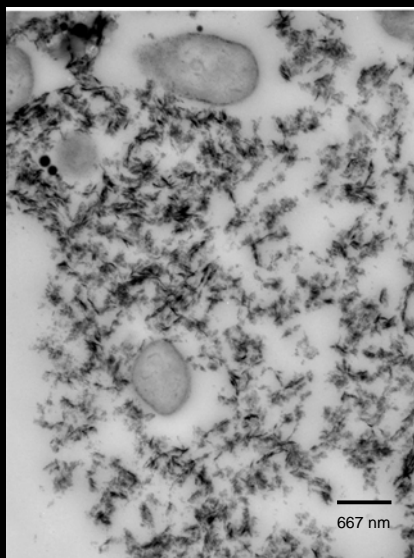
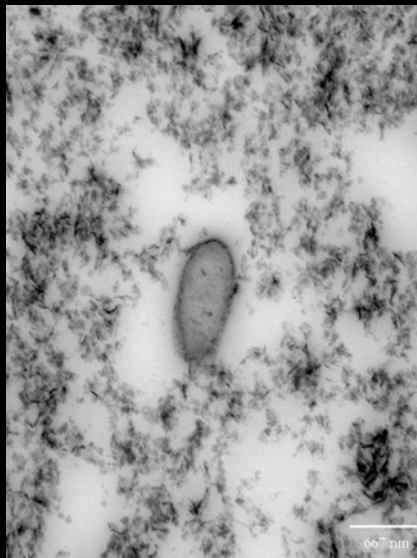


U(VI) added; 12 hr



U(VI) added; 36 hr

Supernatant

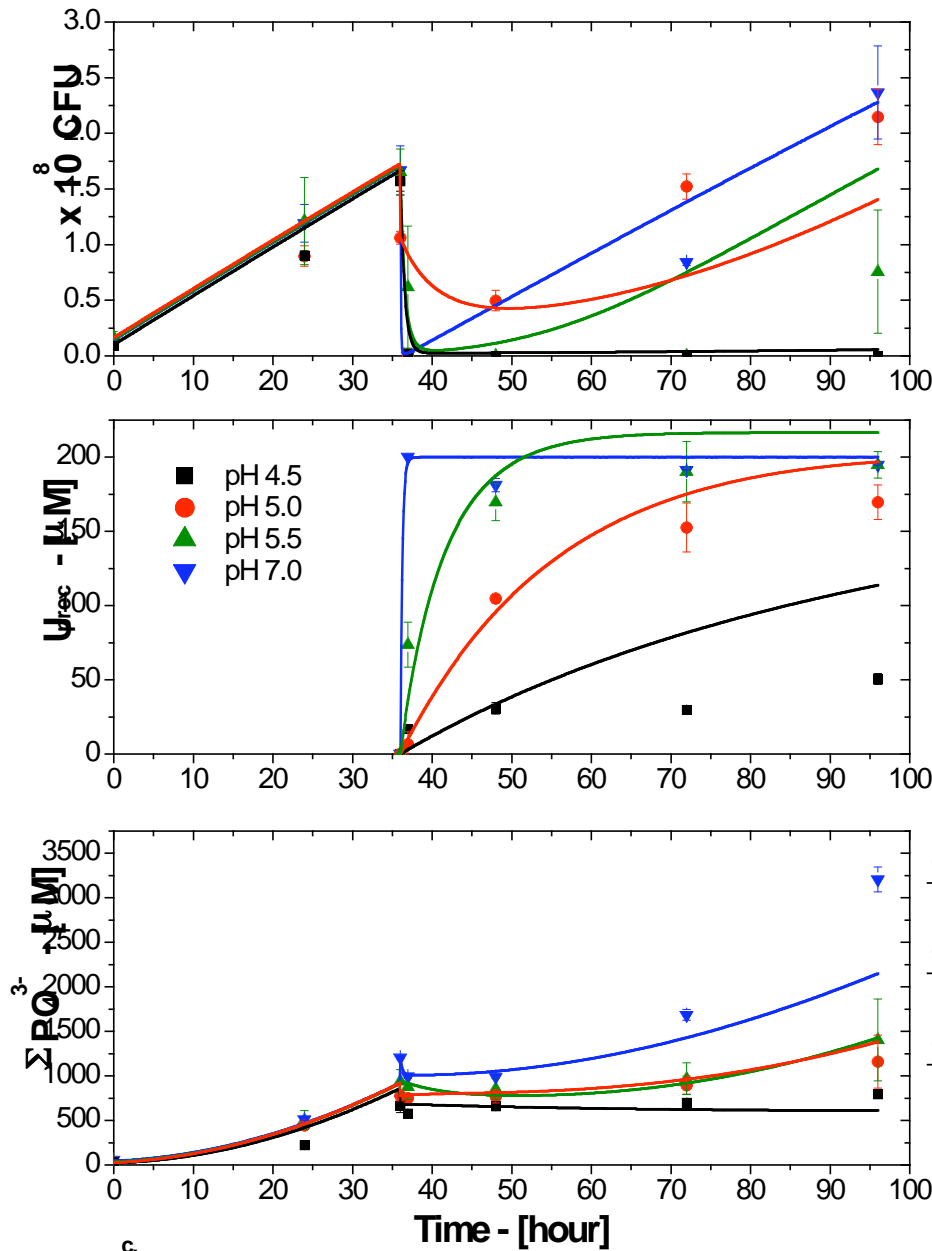


Precipitate

Effect of pH on U(VI) precipitation by *Rahnella* sp.

Proposed Kinetic Model

- *Rahnella* hydrolyzes G3P and grows according to standard Monod kinetics
- Phosphate produced distributes between $H_2PO_4^-$ and HPO_4^{2-}
- The kinetics of precipitation of autunite is influenced by the pH (k_1 and k_2)
- Uranyl is toxic (k_3) and inhibits growth (K_{app}) non-competitively
- *Rahnella* is able to grow back to full rates once U(VI) precipitates



$$\frac{d[UO_2^{2+}]}{dt} = -(k_1[H_2PO_4^-] + k_2[HPO_4^{2-}])[UO_2^{2+}]$$

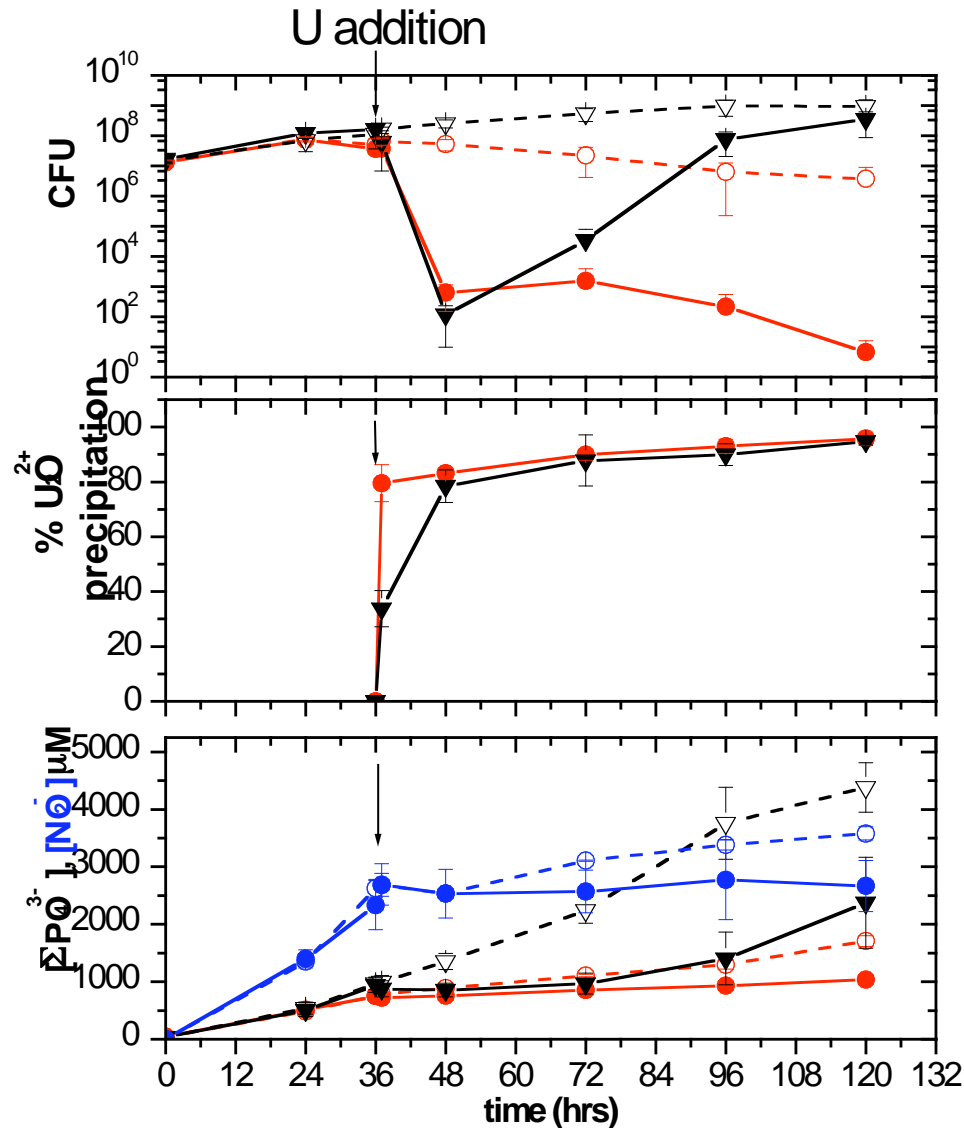
$$\frac{d[\Sigma PO_4^{3-}]}{dt} = -(k_1[H_2PO_4^-] + k_2[HPO_4^{2-}])[UO_2^{2+}] + \frac{R_{max}[G3P]}{K_m + [G3P]}[X]$$

$$\frac{d[X]}{dt} = -k_3f([UO_2^{2+}], [X]) + \frac{\mu_{max}[G3P]}{K_{app} + [G3P]}$$

where $K_{app} = K_s(1 + \frac{[UO_2^{2+}]}{K_i})$

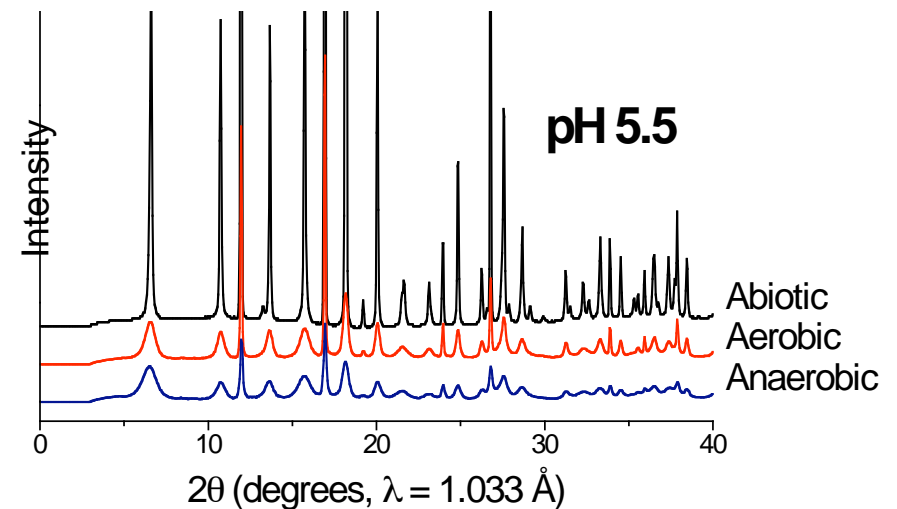


Anaerobic U(VI) precipitation by *Rahnella* sp.



Anaerobic live cells - ○ - without U(VI) - ● - with U(VI)
 Aerobic live cells - ▽ - without U(VI) - ▼ - with U(VI)

Rahnella sp. incubated in simulated anaerobic groundwater with 5 mM NO_3^- at pH = 5.5

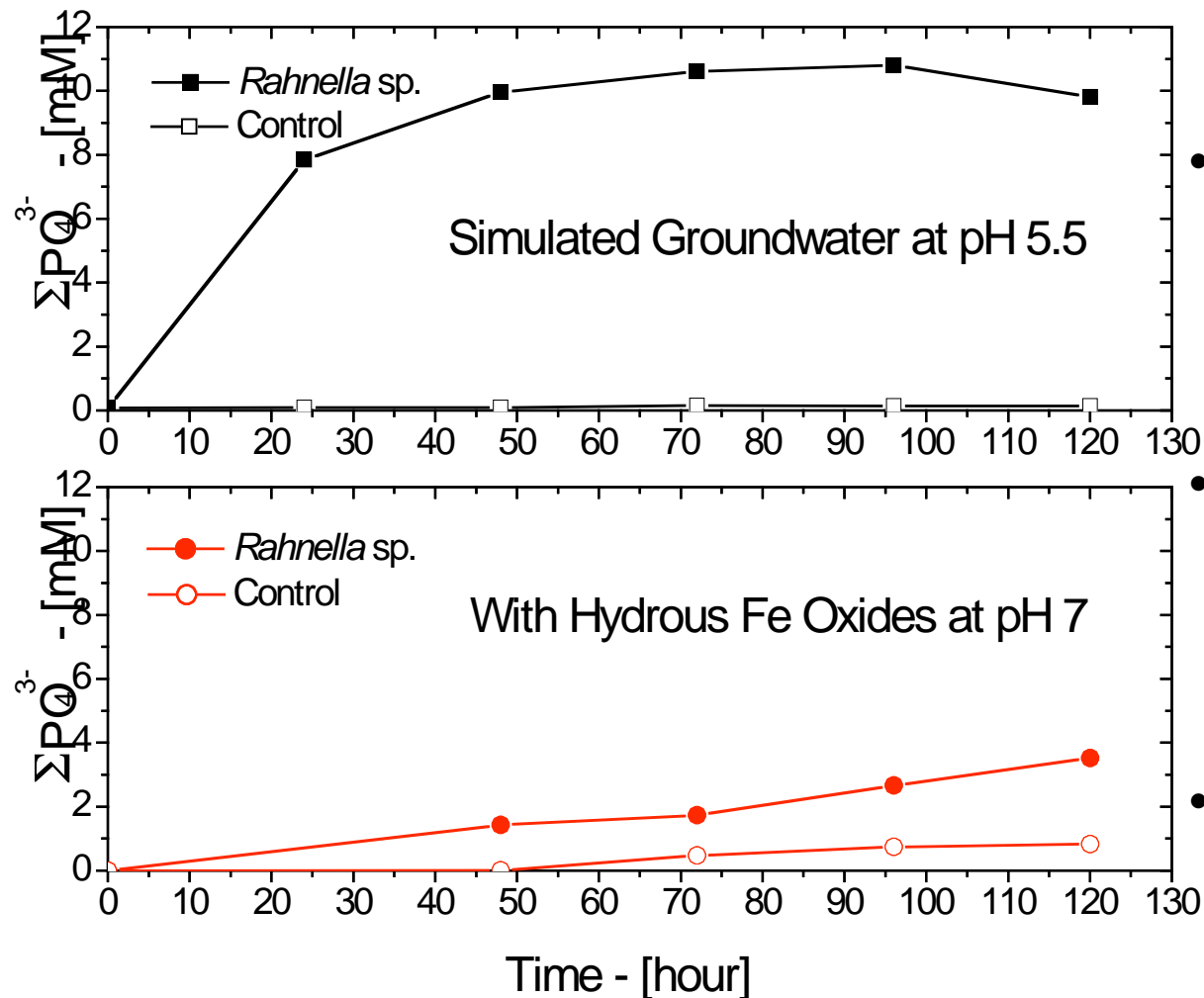


- *Rahnella* sp. respire on nitrate anaerobically.
- G3P hydrolysis is less significant anaerobically.
- U(VI) precipitation is as efficient.
- The combined effect of U(VI) and nitrite appears toxic to the cells.



Effect of solid phase on TPP Hydrolysis

7 mM Glycerol; 3.33 mM TPP with and without Hydrous Fe Oxides (HFO)



- At pH 5.5, *Rahnella* sp. efficiently hydrolyzes TPP

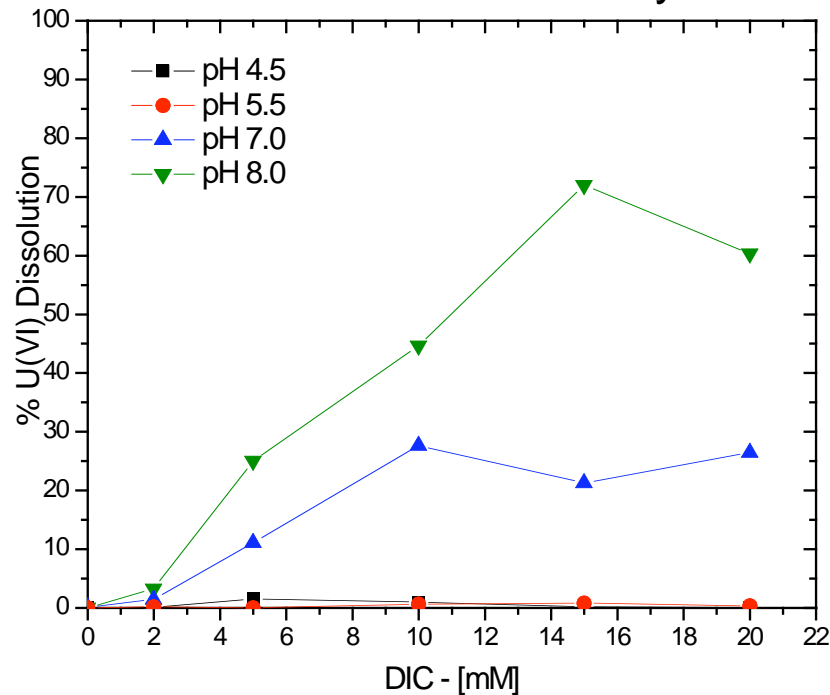
- At pH 7, chemical hydrolysis is enhanced by iron oxides **and** hydrolysis by *Rahnella* sp. is less efficient

- These data suggest that adsorption onto soils may be able to control the release of PO_4^{3-}

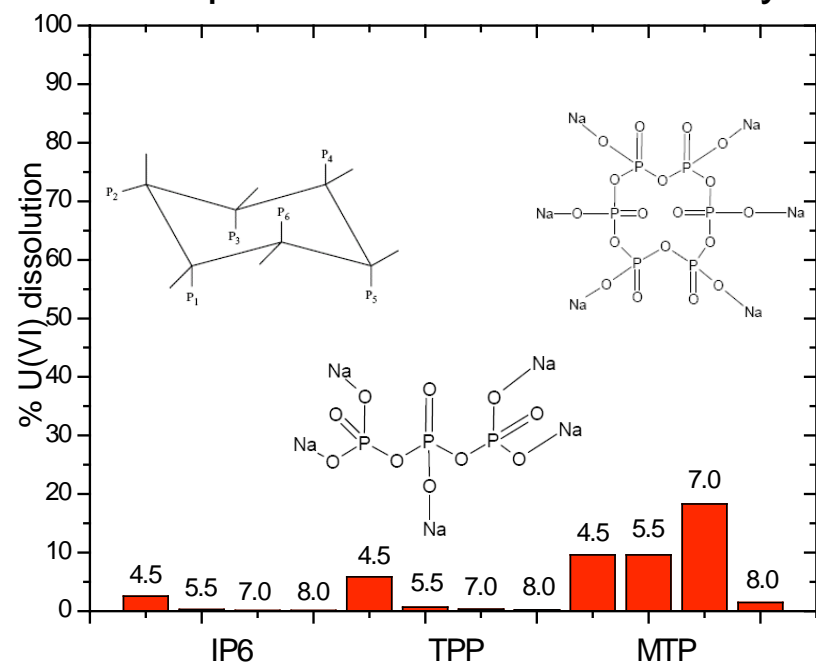


Stability of U(VI) Precipitates

Carbonates after 6 days



Phosphate Sources after 10 days



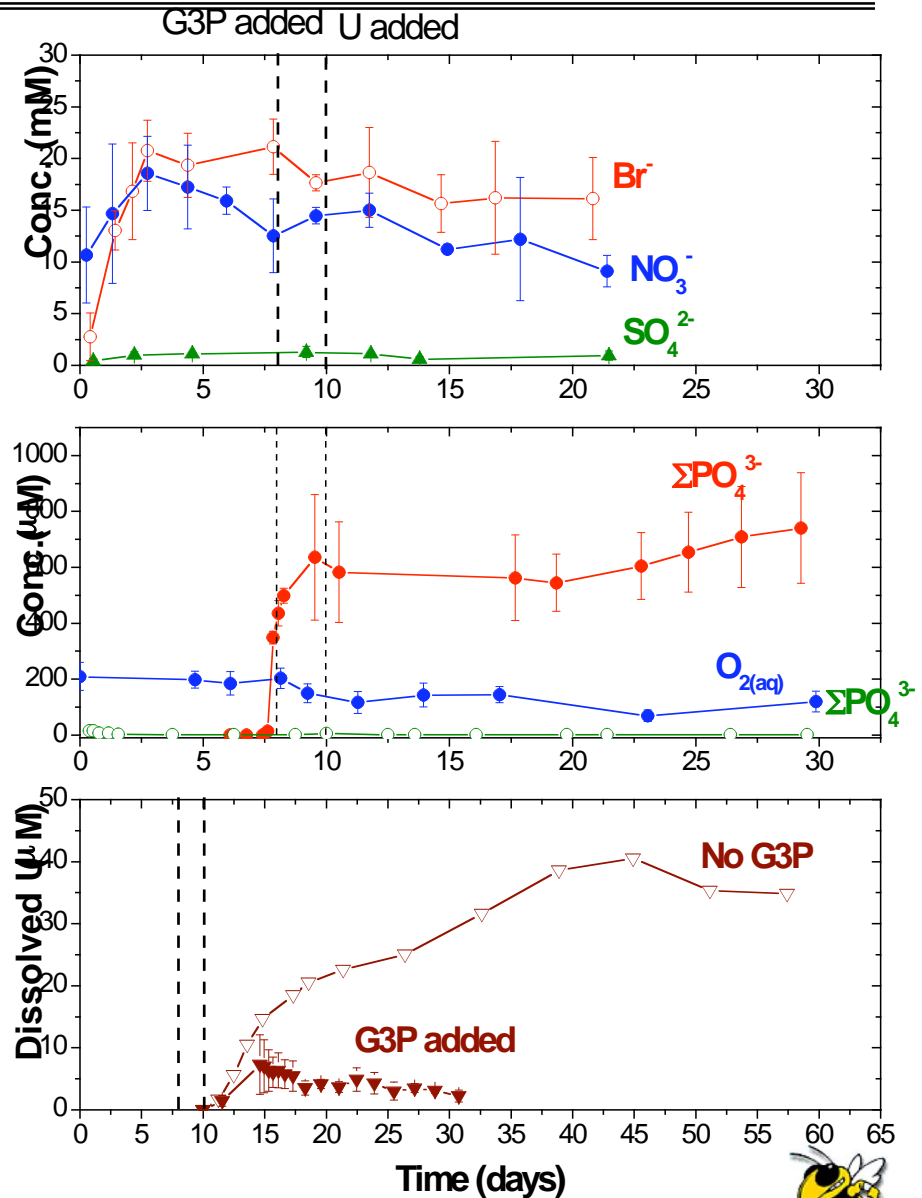
- Below pH 7, autunite precipitates are stable, even in the presence of high concentrations of carbonate
- Organophosphate and polyphosphate sources do not affect the stability of autunites significantly



Small Soil Columns from Area 3

20 mM Br^- ; 15 mM NO_3^- ;
 5 mM G3P added at day 7;
 200 μM UO_2^{2+} added at day 10;
 Simulated groundwater at pH 5.5
 Control: No G3P added

- Natural microbial communities hydrolyze G3P efficiently at pH 5.5
- Reactor remains aerobic even in the presence of 15 mM NO_3^-
- U(VI) is removed efficiently in the presence of G3P. Mechanism remains unknown, but preliminary solid phase extractions show little adsorption.



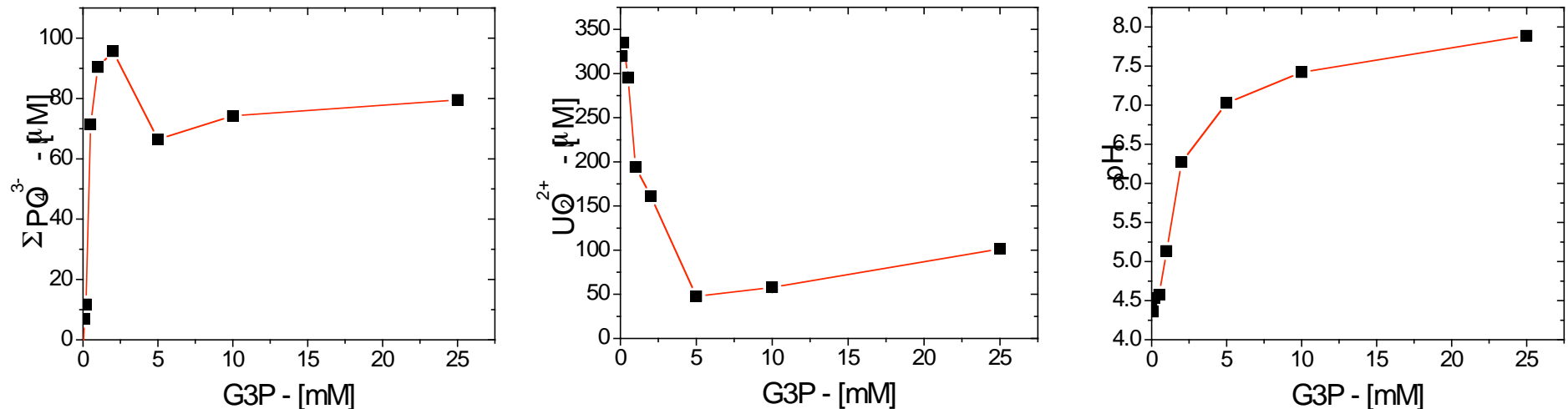
Conclusions

- Biomineralization of U(VI) phosphate is an attractive alternative strategy to the bioreduction of U(VI) in the subsurface
- Metal resistant strains from the ORFRC survive in the presence of U(VI) in a wide range of pHs, probably by actively precipitating autunite minerals using natural organic P sources
- Autunites formed are stable in the pH range 3-8 provided that carbonate concentrations do not exceed 5 mM.
- Soil column experiments reveal that natural microbial communities can hydrolyze 5 mM G3P and remove U(VI) very efficiently at pH 5.5
- Geochemical conditions at ORFRC site are ideal to precipitate U(VI)
- Proposed experiments will investigate:
 - the efficiency of natural organophosphates (e.g., phytate) to biomineralize U(VI)
 - the role of polyphosphate accumulation in some strains as a natural process that could regulate phosphate release when exposed to U(VI)





Abiotic Controls: Effect of G3P Concentration on U(VI) Removal at Steady-State



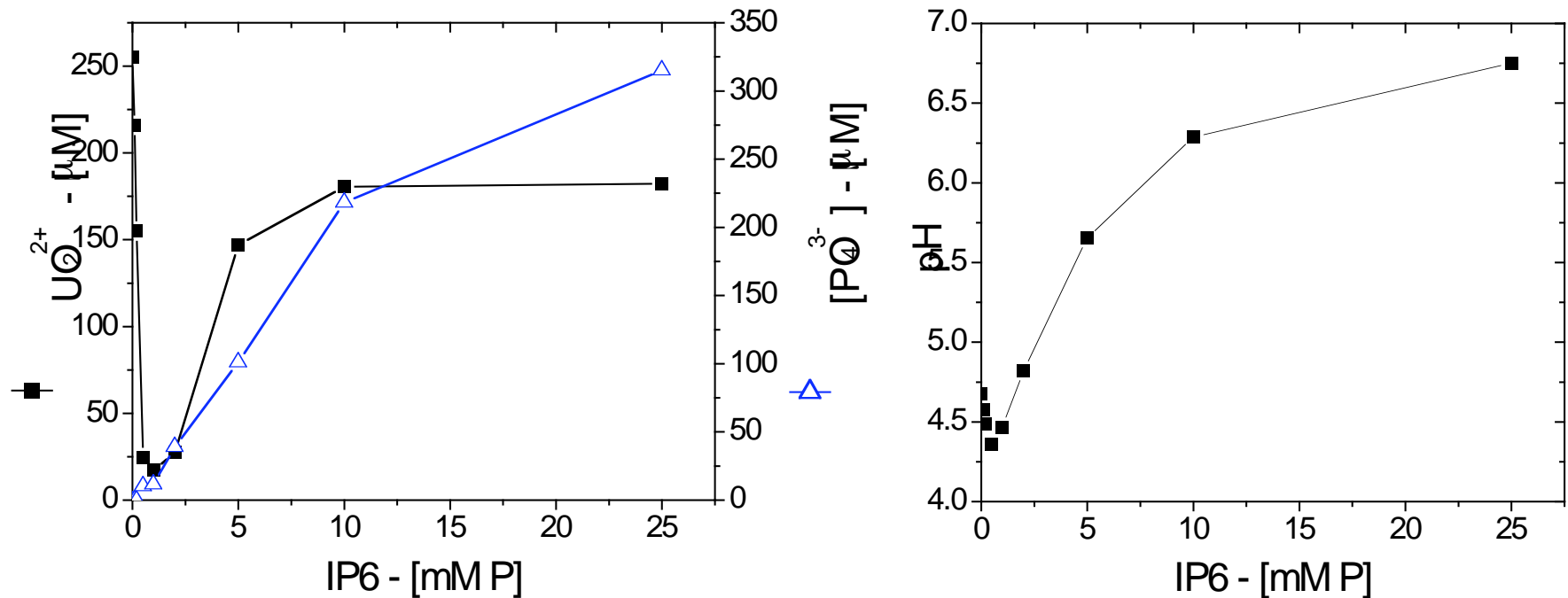
- Background ΣPO_4^{3-} increases asymptotically with G3P concentration
- U(VI) solubility decreases as G3P concentration and pH increase but tend to be more soluble at low pH or high G3P concentration

=> Y9 is not responsible for U(VI) precipitation

- To demonstrate U(VI) biomineralization in aerobic conditions, less chemically labile organo-phosphate compounds must be selected.



Solubility of U(VI) with IP6



- In the presence of IP6 at low concentration, ~90% of UO_2^{2+} precipitates with phosphate
- At high concentration, only ~30% precipitates with phosphate

=> a high concentration of phosphate increases the solubility of uranium phosphate

